Neurological Sciences (2019) 40:2325–2331
https://doi.org/10.1007/s10072-019-03979-0

ORIGINAL ARTICLE

Biallelic pathogenic variants in *TBCD*-related neurodevelopment disease with mild clinical features

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Received: 12 January 2019 / Accepted: 13 June 2019 / Published online: 25 June 2019

Abstract

Background Microtubule dynamics is crucial for neuronal function and survival. The disrupted function of microtubule dynamics would lead to neurodegenerative and neurodevelopmental disorders. Tubulin-specific chaperone D (TBCD) is one of five tubulin co-chaperones acted in assembly and disassembly dynamics of microtubule. The biallelic pathogenic variants of *TBCD* gene were reported to be associated with severe degenerative encephalopathy accompanied with seizures previously.

Results Compound heterozygous variants were identified in three patients from three families. The in silico prediction software and ACMG standards and guidelines proved the pathogenicity of the *TBCD* pathogenic variants. The clinical features of the three patients presented with mild neurodevelopmental manifestations including autism spectrum disorder (ASD) and occasional generalized tonic-clonic seizures (GTCSs) responding well to antiepileptic drugs.

Conclusion Our research expanded the clinical spectrum of *TBCD*-related neurodevelopmental disease which contributed to understanding the genotype-phenotype correlations of the disease.

Keywords Biallelic · Pathogenic variants · *TBCD* · Neurodevelopmental

Background

Microtubule is a dynamic cytoskeleton structure taking part in a wide range of neuronal process such as cell division, polarity, signal transduction, and neuronal morphogenesis [1]. Tubulin-specific chaperone D (*TBCD*) is one of the five tubulin co-chaperones for microtubule subunit α-β-tubulin heterodimer assembly and disassembly. Microtubule behaviors in neuronal proliferation and migration were also relied on normal functions of *TBCD* [2].

The biallelic variants of *TBCD* gene in autosomal recessive hereditary mode could reduce TBCD protein expressions and thus reduce formation of α-β-tubulin heterodimer. The functional alteration of microtubule dynamics caused by *TBCD* led to accelerated polymerization and enhanced stability of microtubule [3]. The defective microtubule dynamics would impair normal neuronal function and survival.

The clinical characteristics of patients with *TBCD* variants were a combination of neurodevelopmental and neurodegenerative manifestations [3]. Common clinical features were severe encephalopathy showing early-onset cortical atrophy, microcephaly, secondary hypomyelination, developmental delay, thin corpus callosum, and progressive spasticity [1].

Since 2016, Flex et al. reported seven patients presented with encephalopathy carrying biallelic *TBCD* variants exhibiting autosomal recessive hereditary pattern, only seven researches had reported *TBCD*-related neurodevelopmental and neurodegenerative diseases [1–7]. The clinical features of the patients by far manifested with severe clinical features as described above. Our analysis found three patients presenting with more benign clinical features than previous reported ones which widened the clinical spectrum of *TBCD*-related neurodevelopmental disease.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-019-03979-0) contains supplementary material, which is available to authorized users.

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Methods

Patient recruitment

The three patients and their corresponding parents included in our research all had informed consents, and the study was approved by the Ethics Committee of Xiangya Hospital. Leukocyte DNA was extracted from peripheral blood by phenol chloroform method. Clinical information was collected by experienced neurologists. Electroencephalogram (EEG) and magnetic resonance imaging (MRI) were performed on each patient. No inborn metabolism errors were reported by patients. The diagnosis of autism spectrum disorder (ASD) was according to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria [8].

Whole-exome sequencing and bioinformatic analysis

DNA from the patients were sequenced and analyzed using whole-exome sequencing (WES) approach. HiSeq2500 system (Illumina, San Diego, CA, USA) with a mean depth of 100× were used to sequence the DNA fragments.

A preliminary handling (data alignment and filter) of WES data were done following protocols of Wang [9]. Then, the data was annotated by ANNOVAR for following analyses [10]. As the variant inherited in autosomal recessive trait, biallelic variants (homozygous or compound heterozygous variants) were prioritized. Public databases (ESP6500, 1000 Genomes, ExAC, GnomAD) were used to filter variants with frequencies above 0.001. Four softwares (Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen-2), Combined Annotation Dependent Depletion (CADD), and Mutation Taster) were used to predict pathogenicity of variations. The international guidelines of the American College of Medical Genetics (ACMG) guidelines were used to interpret the pathogenicity of variants [11].

Sanger sequencing was conducted on patients' DNA of the same samples to validate results of WES and on parents' DNA to validate hereditary patterns. Primers were designed according to pathogenic variants found in WES in patients, respectively, and polymerase chain reaction amplifications were conducted for Sanger sequencing.

Results

Pathogenic variants in patients

We identified three compound heterozygous variants of *TBCD* in the three patients (Table 1, Figs. 1). The variants demonstrated extremely rare frequencies in GnomAD, ExAC, 1000Genomes, ESP6500 databases. As shown in Table 1, all of the pathogenic variants in *TBCD* were predicted pathogenic by ACMG guidelines.

Clinical presentations in patients

Patient 1 showed initial seizures in 1 year of age. Seizures were occasional generalized tonic-clonic seizures (GTCSs) with a frequency of about three to four times per month. The treatment with oxcarbazepine and levetiracetam were effective. Interictal EEG revealed a large number of spike waves; spike and slow waves were distributed in the bilateral central top, middle, and left and right temporal regions during the sleep period

Table 1 The pathogenic variants of *TBCD*

Patient no.	Pathogenic variant (base alteration)	Pathogenic variant (amino acid alteration)	Pathogenic variant types	SIFT	Polyphen-2	MutationTaster	CADD	ACMG
1	c.3365C/T (Fig. 1)	p.P1122L	Nonsynonymous	Tolerable	Probably_damaging	Disease_causing	Damaging	Pathogenic
	c.1739G/A (Fig. 2)	p.R580Q	Nonsynonymous	Damaging	Probably_damaging	Disease_causing	Damaging	Pathogenic
2	c.230A/G (Fig. 3)	p.H77R	Nonsynonymous	Damaging	Probably_damaging	Disease_causing	Damaging	Pathogenic
	c.907C/T (Fig. 4)	p.R303*	Stopgain	–	–	Disease_causing_automatic	Damaging	Pathogenic
3	c.2953C/T (Fig. 5)	p.R979C	Nonsynonymous	Damaging	Probably_damaging	Disease_causing	Damaging	Pathogenic
	c.3550C/T (Fig. 6)	p.Q1184*	Stopgain	–	–	Disease_causing_automatic	Damaging	Pathogenic

No. number, *SIFT* Sorting Intolerant From Tolerant, *PolyPhen-2* Polymorphism Phenotyping v2, *CADD* Combined Annotation Dependent Depletion, *ACMG* The international guidelines of the American College of Medical Genetics

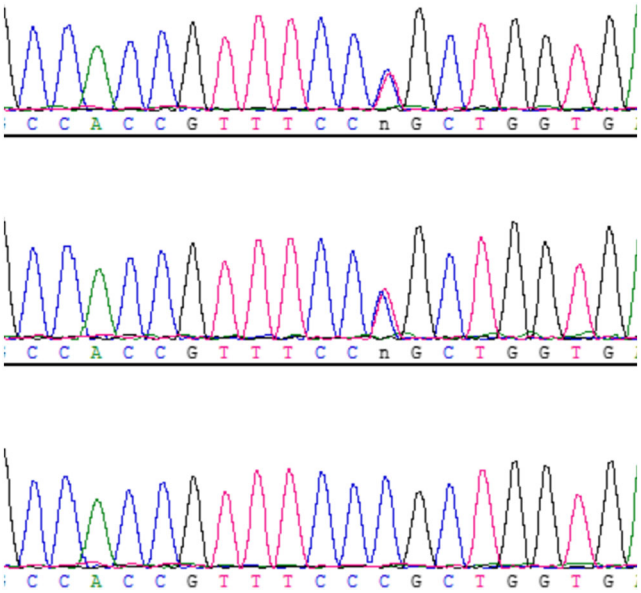

Fig. 1 Sanger sequencing confirmed the biallelic variant c.3365C/T in three patients. The orders of the variants referred to Table 1

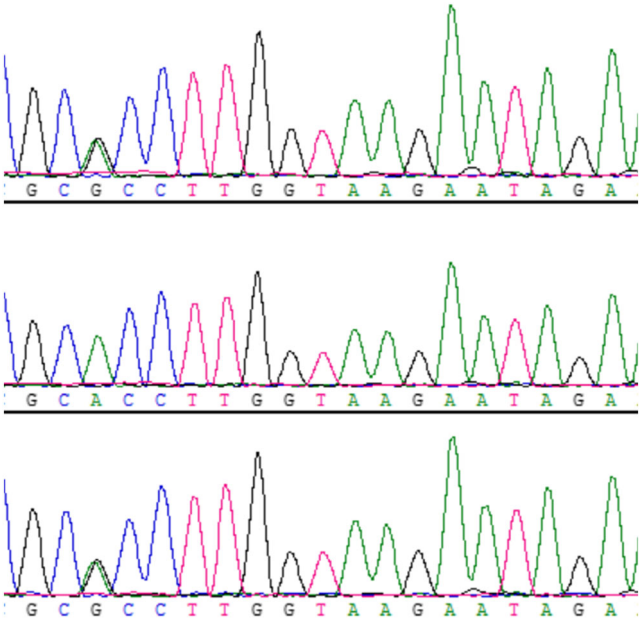

Fig. 3 Sanger sequencing confirmed the biallelic variant c.230A/G in three patients. The orders of the variants referred to Table 1

(Fig. 7). MRI showed myelination delay reflected by abnormal signal in the white matter in occipital lobe (Supplementary Figs. 1 and 2). At the age of 4 months, her parents noticed that although the patient managed to lift her head and chest, there was decreased smiling and babbling. At the age of 6 months, she needed some support to sit and did not have the same curiosity as infant of the same age in trying to get things around. When first visited our outpatient at the age of 1 year and 4 months, we need to hold her hands sometimes when walking unstably. Neurological examination was normal except for dystonia (Table 2).

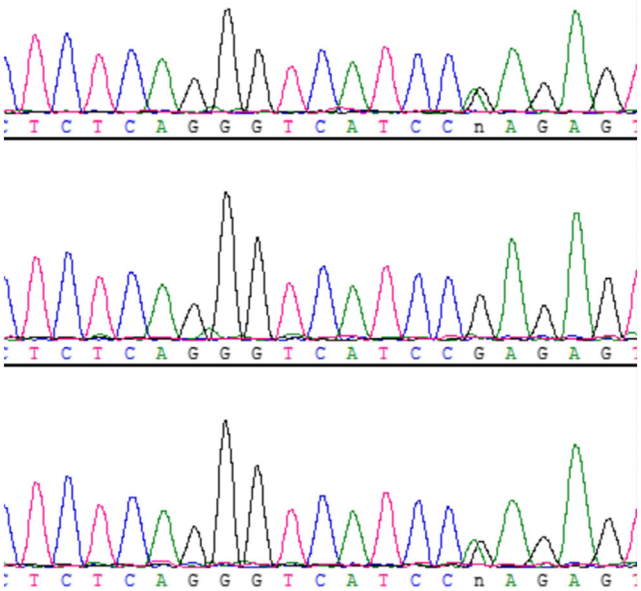

Fig. 2 Sanger sequencing confirmed the biallelic variant c.1739G/A in three patients. The orders of the variants referred to Table 1

Patient 2 reflected febrile seizures. She showed initial seizures in 6 months of age. Seizures observed were occasional GTCS induced by fever and were in good response to antiepileptic drugs such as oxcarbazepine. Interictal EEG revealed distribution of low amplitude spike waves in midline, bilateral central top, and frontal regions during sleep (Fig. 8). MRI showed myelination delay reflected by abnormal signal in the white matter in occipital lobe (Supplementary Figs. 3 and 4). The patient could almost reach the developmental milestones of infant. At the age of 6 months, she could bring nearby things to mouth and begin to sit without support. At 1 year of age, she could respond to simple words, say simple words, and stand with some support. When first visited our outpatient at the age of 1 year and 8 months, he could stand, jump, and turning book pages. Neurological examination was normal (Table 2).

Patient 3 showed ASD when he was 3 years and 2 months when visiting our outpatient for the first time. He did not communicate with his friends and did not eat by himself. He walked on tiptoe and did not play with strangers. In most time of the day, he liked to turn around, rub his hands, and row toys without being able to play with them. He could express simple requirements such as peeing in pants. When asked his name in the outpatient, he had few response and covered his face with his mother's hands. All questions had few answers.

Table 2 The clinical presentations of patients with *TBCD* pathogenic variants

	Patient 1	Patient 2	Patient 3
Gender	Female	Female	Male
Age at clinical presentation	1 year 4 months	1 year 8 months	3 years 2 months
Age at onset (epilepsy)	1 year	6 months	–
Head circumference (cm)	46	47	48.5
Other dysmorphia	–	–	–
Intellectual disabilities	Slight delay	Nearly normal	
Seizures	GTCS	GTCS	–
Hypotonia	+	–	–
Other nervous or muscular abnormalities	–	–	–
Skeleton dysfunctions	–	–	–
Respiratory dysfunctions	–	–	–
Dysfunctions in autonomic system	–	–	–
ASD	–	–	+
MRI dilations of ventricles	–	–	–
MRI thinning of the white matter and corpus callosum	–	–	–
MRI atrophy of cortex and cerebellum	–	–	–
EEG	Epileptic EEG	Epileptic EEG	–

ASD autism spectrum disorder, *MRI* magnetic resonance imaging, *EEG* electroencephalogram

At the age of 6 months, he could babbling and respond to his name. He could sit without support and roll back and forth. At the age of 1 year, he could stand without support and say simple words. At the age of 3 years and 2 months, he could read, dress himself, and even walk up the stairs. MRI and neurological examination was normal (Table 2, Supplementary Figs. 5 and 6).

Discussion

In our reports of three patients carrying *TBCD* pathogenic variants, we identified a novel clinical feature—ASD—in one patient and two mild clinical features in two patients. Our research identified biallelic pathogenic variants in *TBCD*-related neurodevelopment disease with mild clinical features for the first time. In detail, two patients had occasional GTCS with good response to antiepileptic drugs. Patient 3

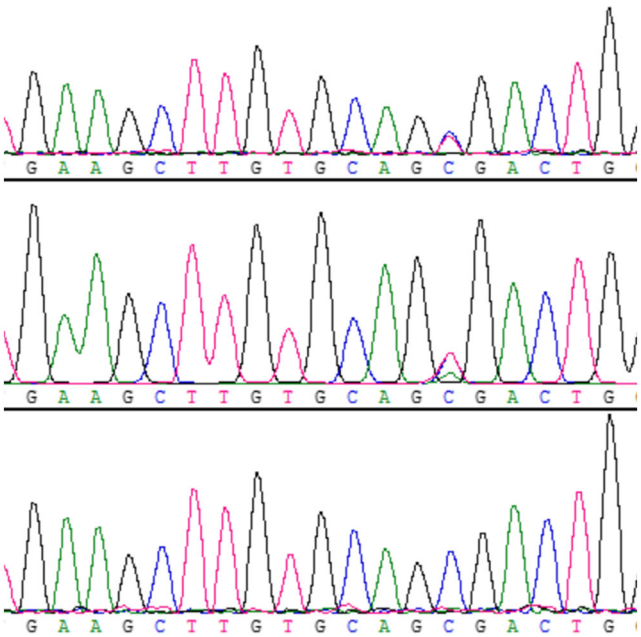

Fig. 4 Sanger sequencing confirmed the biallelic variant c.907C/T in three patients. The orders of the variants referred to Table 1

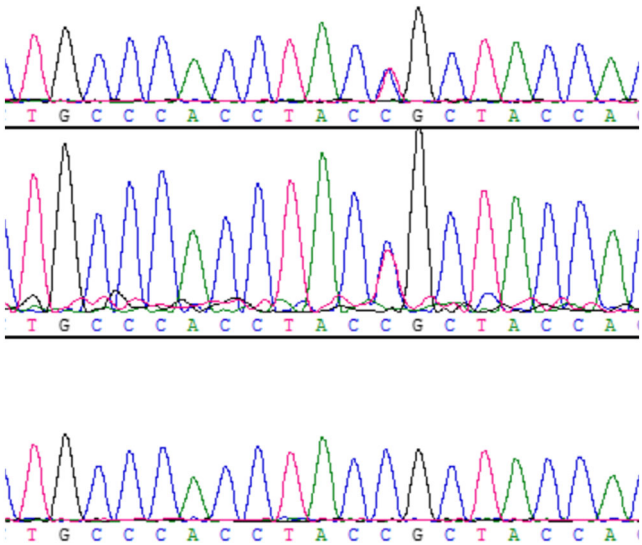

Fig. 5 Sanger sequencing confirmed the biallelic variant c.2953C/T in three patients. The orders of the variants referred to Table 1

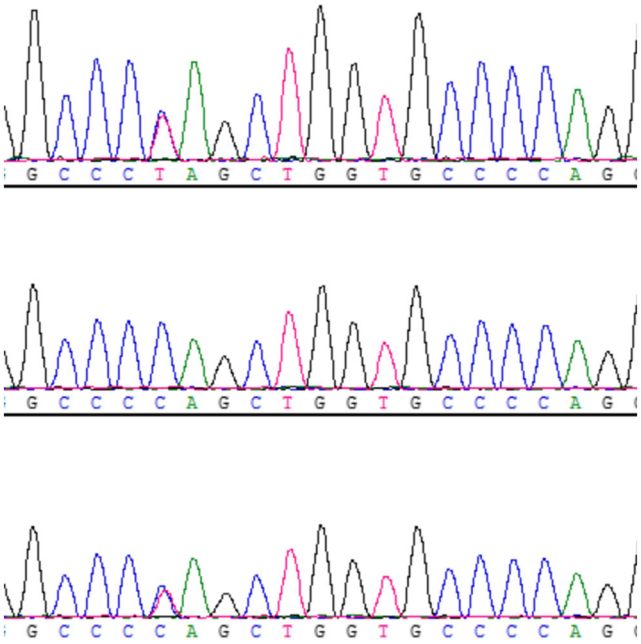

Fig. 6 Sanger sequencing confirmed the biallelic variant c.3550C/T in three patients. The orders of the variants referred to Table 1

only presented with ASD without any other neurodevelopmental disorders or seizures.

The affected patients showed mild disease course without reported severe clinical manifestations including severe intellectual disabilities, microcephaly, skeleton dysfunctions, respiratory dysfunctions, and dysfunctions in autonomic system [1–7]. The MRI of the three patients did not present with previous reported severe clinical features as dilations of ventricles, thinning of the white matter and corpus callosum, and atrophy of cortex and cerebellum. Our research widened the clinical spectrum of *TBCD*-related developmental diseases.

As microtubule dynamics is essential for a wide variety of cellular processes, the normal function of one of the co-chaperon TBCD mediating the process is crucial. The pathogenic variants of *TBCD* will affect the TBCD level and function, disrupting the normal function of neurons. Up till now, only seven researches reported *TBCD*-related neurodevelopmental and neurodegenerative diseases [1–7]. The clinical features of *TBCD*-related neurodevelopmental and neurodegenerative diseases were severe as listed in Table 2 items. However, our research discovered a more benign clinical manifestation than previous reports which indicates a possible genotype-phenotype correlation of *TBCD*-related epilepsy.

Few researches discussed the mechanisms underlying the genotype-phenotype correlations of *TBCD*. Miyake et al. hold the opinion that such correlation was due to the level of residual protein dysfunction caused by the pathogenic variants [6]. As to the specific clinical features observed in our study, previous researches hold the opinion that the mechanism underlying *TBCD*-related epilepsy was due to the change of TBCD protein levels or function. The disruption of α/β-tubulin heterodimer assembly and disassembly equilibrium would cause affected polymerization and stability of microtubule. The

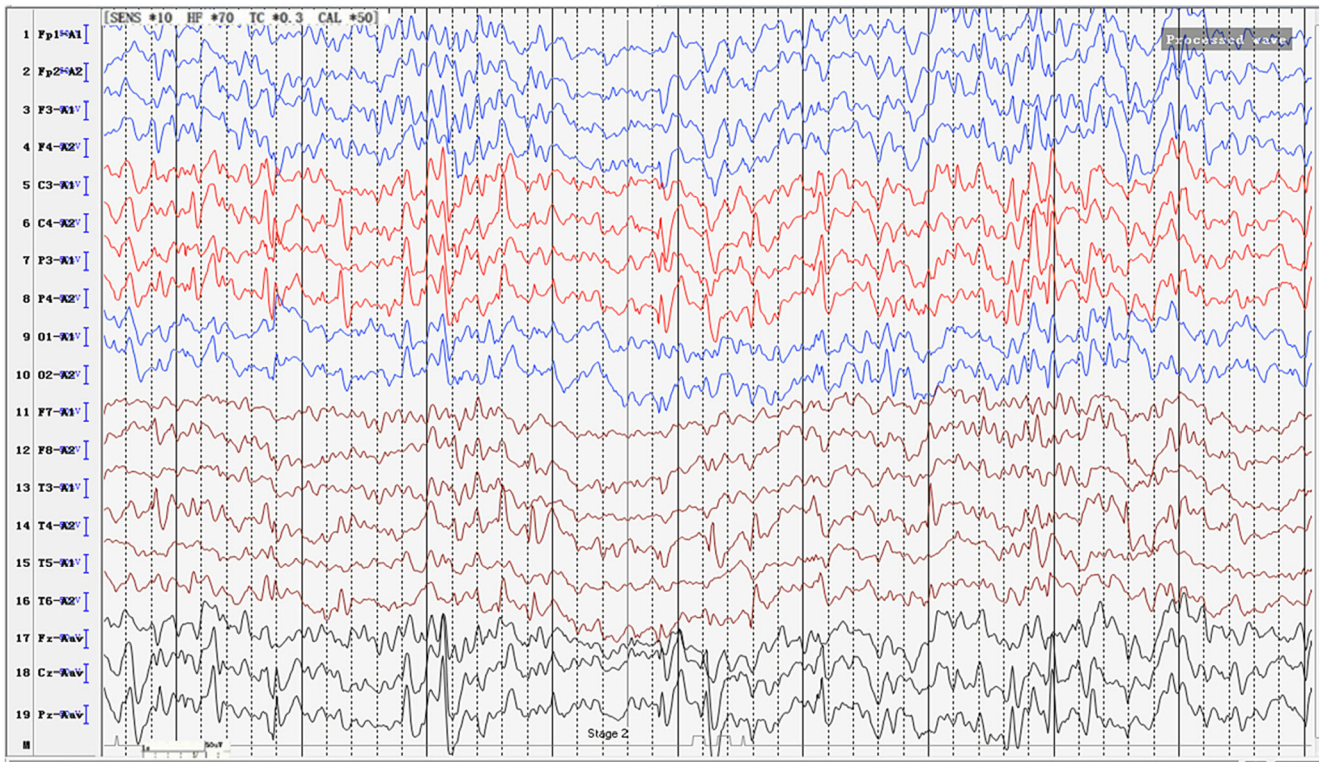

Fig. 7 EEG of patient 1

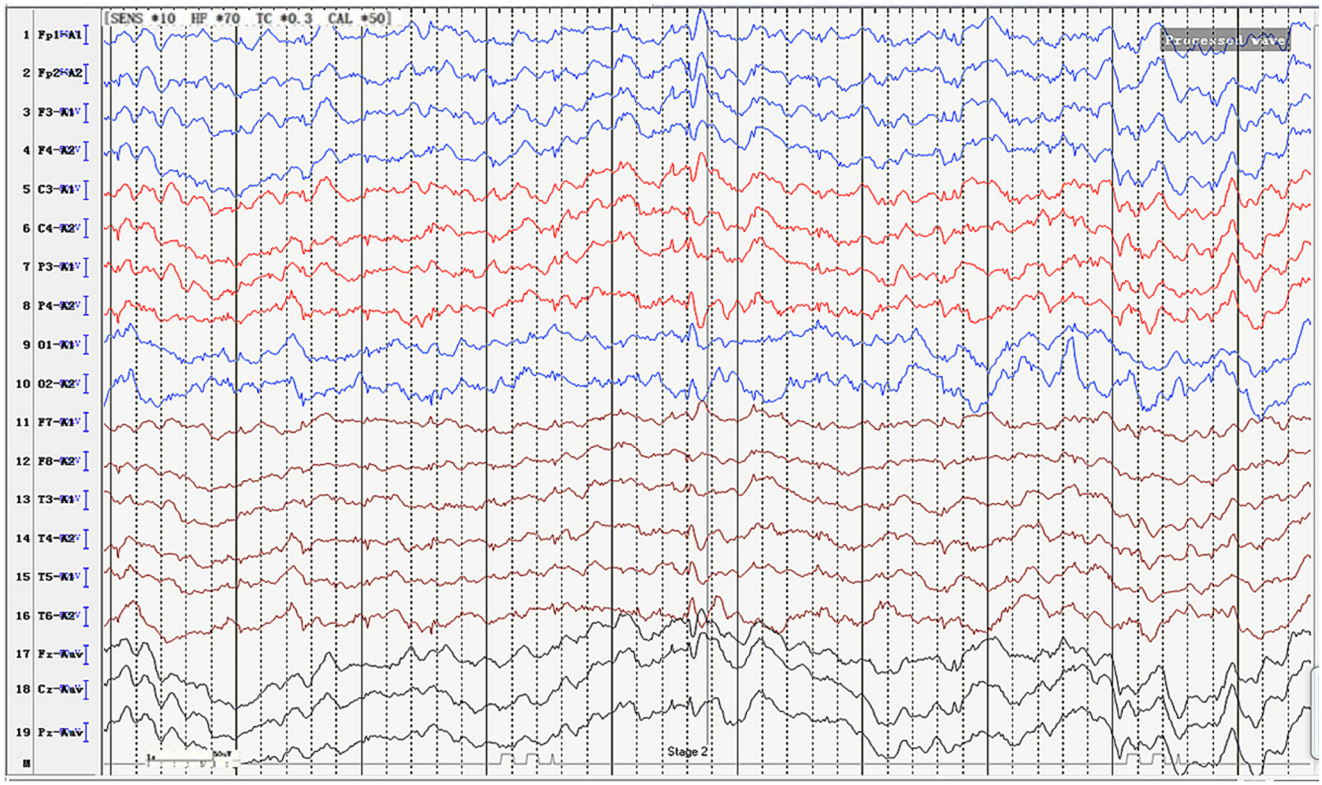

Fig. 8 EEG of patient 2

biallelic pathogenic variants of *TBCD* could cause dysregulation of microtubule dynamics by affecting β-tubulin folding, microtubule polymerization, and depolymerization [3]. The depolymerizing of microtubule was associated with chronic epilepsy via mediating postsynapsis in interneuron activation [12].

ASD was a novel clinical phenotype of *TBCD*-related disorders observed by our research. The main clinical features of ASD are its deficit in social interaction and repetitive and restrictive behaviors [13]. Previous research had found that autism susceptibility candidate 2 gene (*AUST2*) could interact with microtubule-interacting proteins (MAPs) and was associated with ASD [14]. MAPs could adjust microtubule dynamics and played an important role in ASD pathogenesis by modulating the neuron and brain development processes [13]. In our research, we identified *TBCD* pathogenic variants in an ASD patient which also functioned in microtubule dynamics and associated with the disease. Furthermore, one of the most important gene linked to ASD—*CHD8*—could bind to *TBCD* and regulate its function (https://gene.sfari.org/database/human-gene/Chd8#pin-tab). And *TBCD* is a postsynaptic density gene which functioned in brain signal processing and transmission. Mutations of such genes were reported to be major causes of psychiatric disorders including autisms, intellectual disabilities, and schizophrenia [15].

Conclusion

In conclusion, the mild clinical features including a novel clinical feature—ASD—caused by biallelic pathogenic variants of *TBCD* were found in our research. It expanded the clinical spectrum of *TBCD*-related epilepsy which contributed to understanding the genotype-phenotype correlations of the disease.

Funding This work was supported by grants from the National Natural Science Foundation of China (No. 81801136) and China Postdoctoral Science Foundation.

Compliance with ethical standards

Ethics approval and consent to participate The parents included in our research all had informed consents, and the study was approved by the Ethics Committee of Xiangya Hospital.

Consent for publication We have obtained consent to publish from the participants' parents to report individual patient data.

Availability of data and material Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests The authors declare that they have no competing interests.

Abbreviations ASD, autism spectrum disorder; GTCS, generalized tonic-clonic seizures; TBCD, tubulin-specific chaperone D; EEG, electroencephalogram; MRI, magnetic resonance imaging; DSM, American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders; WES, whole-exome sequencing; SIFT, Sorting Intolerant from Tolerant; PolyPhen-2, Polymorphism Phenotyping v2; CADD, Combined Annotation–Dependent Depletion; ACMG, the international guidelines of the American College of Medical Genetics; MAPs, microtubule-interacting proteins

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